Crystallization and Preliminary X-Ray Analysis of *Sau3*AI/E64A Mutant Protein

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Abstract: *Sau3*AI is a type II restriction endonuclease that recognizes the palindromic sequence 5'–GATC-3' and cleaves 5' to G residue on each strand. The E64A mutant full length protein was cloned and expressed in *Escherichia coli*. The purified $(His)_6$-tagged protein has monomer and dimer fraction and was crystallized by the hanging-drop vapor-diffusion technique. The dimer protein crystals can diffract to 3.0Å resolution and the monomer protein crystals can diffract to better than 2.8Å resolution. One completed dataset has been collected and it shows that the monomer orthorhombic *Sau3*AI/E64A crystal is in space group C2221 with unit cell parameters (69.44, 197.60, 191.46, 90, 90, 90) and contains two molecules in one asymmetric unit.

Keywords: *Sau3*AI endonuclease, crystallization, preliminary X-ray analysis.

INTRODUCTION

*Sau3*AI endonuclease is a 56KD protein containing 489 amino acids. It is classified as type IIe restriction enzyme and binds two copies of recognition sequence for cleavage function [1]. The N-terminal of *Sau3*AI is thought as the catalytic domain and the C-terminal of *Sau3*AI is thought as the effector domain.

Based on sequence alignment, the N-terminal *Sau3*AI shows the same active amino acids as MutH [2]. The glutamic acid at position 43, aspartic acid at position 57, glutamic acid at postion 64 and lysine at position 66 may take responsibility to bind and digest DNA through two magnesium as in MutH and some other type II restriction enzymes [3]. Our data showed that the enzyme activity decreased or was totally lost after mutated these amino acids to alanine (data is in prepared).

The structure and character of *Sau3*AI endonuclease is still unclear. To investigate its 3D-structure and function, we expressed the *Sau3*AI E64A mutant in *E. Coli* and crystallized it after purification. In this paper we report the preliminary X-Ray results of *Sau3*AI crystals.

MATERIALS AND METHODS

Expression and Purification

Plasmid pWY1440 containing full length *Sau3*AI gene from *Staphylococcus aureus* 3AI in pET15b was a gift from Dr. Wei Yang (NIDDK/NIH, USA). It has an E64A / GAA→GCA mutation and silence mutations at the C terminal end as shown before [4]. It also has a (His)6 tag and a thrombin digestion site at the N-terminal. The plasmid was transferred to Rossetta plysS competent cell and then expressed at 25°C for 12hr with 0.2mM IPTG induction.

Cells were harvested and resuspended in buffer of 20mM Tris-HCl pH8.0 / 400mM NaCl / 1.4mM 2-mercaptoethanol /1mM PMSF. After medium power sonication for 60s, 3 times at 4°C to lysis the cells, the supernatant was got by centrifuging at 16,000rpm for 30 min and was loaded on Ni-NTA. *Sau3*AI/E64A protein was eluted from Ni Column by using a buffer containing 20mM Tris-HCl pH8.0 / 400mM NaCl /1.4mM 2-mercaptoethanol /300mM imidazole. Then the sample was diluted using 5 times volume of 20mM Tris-HCl pH8.5 / 1mM DTT / 5% glycerol and loaded on an Amersham HiTrap Q HP column for further purification. During a gradient to 20mM Tris-HCl pH8.5 / 1mM DTT / 400mM KCl / 5% Glycerol, two elution peaks corresponding to *Sau3*AI protein monomer and dimer respectively appeared (data not shown). The two peaks were concentrated separately in the buffer of 20mM HEPES pH7.2 / 200mM KCl / 5mM MgCl2 / 1mM EDAT / 1mM DTT / 5% glycerol using Millipore Amicon Ultra 30KD. Fresh protein without cleavage of the 6 His tag was concentrated to over 20mg/ml and was diluted to 10mg/ml for crystallization.

Crystallization and X-Ray Data Collection

Crystallization was carried out by hanging drop diffusion method at 4℃. For the *Sau3*AI/E64A protein monomer, plate-like crystals appeared overnight in hanging drops equilibrated over a reservoir solution consisting of 16% PEG 8K/ 0.1 M sodium acetate pH 4.6/ 0.2M CaCl2 (Fig. **1a**) and were frozen in cryo-solution 20%PEG 8K / 20% glycerol / 0.1 M sodium acetate pH 4.6 / 100 mM KCl / 5mM MgCl2 /1 mM EDTA / 1 mM DTT. X-ray diffraction data were exposed 20 min for each image and were collected at 100 K on a MAR345dtb image plate detector (MarResearch, Germany) with a rotating-anode X-ray generator (copper anode; RA-Micro007, Rigaku, Japan). For *Sau3*AI/E64A monomer crys-

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0929-8665/07 $50.00+.00

tal in this condition, one completed data set was obtained by collecting 60 images with 1°oscillation. The data was processed using AUTOMAR v.1.4 (MAR Research GmbH). The monomer *Sau3*AI/E64A mutant crystals were also found in the condition of 15% PEG 3350/ 0.1 M sodium citrate tribasic dihydrate pH 5.0/ 0.2M di-Ammonium hydrogen citrate (Fig. **1b**) and were flash-frozen with additional 20% glycerol. In the case of *Sau3*AI/E64A protein dimer, 3D crystals were obtained in 6% -8% PEG8K / 0.1M HEPES pH7.5 (Fig. **1c**) and were gradually transferred to the cryoprotectant solution with additional 20% glycerol. The dimer *Sau3*AI/E64A mutant proteins were also crystallized in 2M (NH4)2SO4 / 0.1M sodium citrate tribasic dihydrate pH5.6/ 0.2M K/Na Tartrate after 12h and finally grew to about 0.4X0.2X0.2 mm (Fig. **1d**) and were frozen with additional 20% glycerol and 20% MPD.

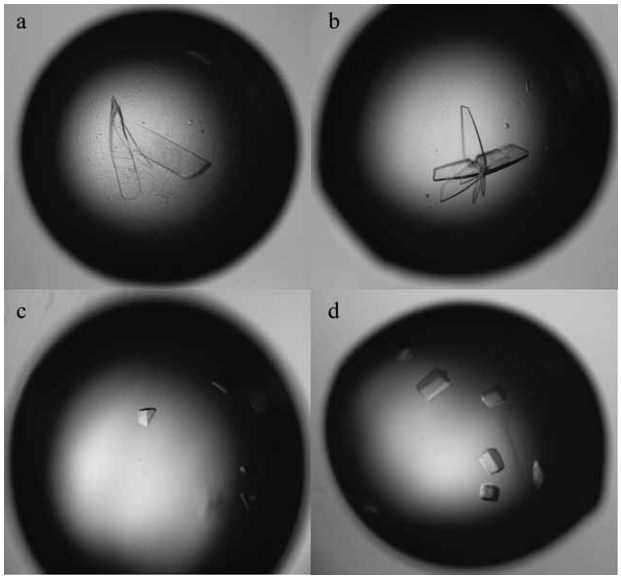

Figure 1. The crystals of *Sau3*AI E64A mutant monomer (a and b) and dimer (c and d) in different conditions as described.

RESULTS AND DISCUSSION

The preliminary crystallography results of *Sau3*AI/E64A monomer crystals from condition of 16% PEG 8K/ 0.1 M sodium acetate pH 4.6/ 0.2M CaCl2 were shown in Table **1**. In the diffraction image it can be found that some spots reached the resolution of 2.2 Å, but the completeness was only 79.7% after integrated these 60 images to 2.22 Å. For the *Sau3*AI/E64A monomer crystals in another condition and the dimer crystals, the best resolution was found at 3.0 Å and the data were not collected.

The role of C-terminal fragment of *Sau3*AI in DNA binding and cleavage specificity is still unclear. The plate-like crystal of the C-terminal *Sau3*AI fragment was previously reported to belong to orthorhombic space group $P2_12_12_1$ with the cell dimension of a = 34.75, b = 76.82, c = 123.59Å and contain one molecule per asymmetric unit [4]. We are now growing seleno-methionine-derivated crystals of *Sau3*AI/E64A monomer to determine the phase and to solve the structure.

Table 1. Crystallographic Data of *Sau3*AI/E64A Protein

Space Group	C2221
Unit Cell parameters (Å)	69.44, 197.60, 191.46
Resolution limits[1] (Å)	30.00-2.80 (2.90-2.80)
Observed reflections	142974
Unique reflections	60750
$\langle I/\sigma(I)\rangle$ [1]	7.1 (1.5)
Rmerge[1] (%)	4.21 (32.25)
Mosaicity	0.17
Completeness[1] (%)	94.9 (95.3)
No. of subunits per asymmetric unit	2
Solvent content (%)	56.1

[1] The values in parentheses are for the highest resolution shell.

ACKNOWLEDGEMENTS

The authors thank Prof. Maikun Teng (University of Science and Technology of China, P.R China) for the help of data collection and X-ray machine assistant. Grants (No. 30600107, 30670499, 30500113 and 30400064) from the National Natural Science Foundation of China and the funding from Shanghai Institute of Applied Physics, Chinese Academy of Sciences, are gratefully acknowledged.

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Received: February 02, 2007 Revised: March 23, 2007 Accepted: March 30, 2007